Environmental Health Perspectives
Vol. 66, pp. 149-153, 1986

Prevalence of IgE Antibodies to Grain and Grain Dust in Grain Elevator Workers

by Daniel M. Lewis,* Peggy A. Romeo,* and Stephen A. Olenchock*

IgE-mediated allergic reactions have been postulated to contribute to respiratory reactions seen in workers exposed to grain dusts. In an attempt better to define the prevalence of IgE antibodies in workers exposed to grain dusts, we performed the radioallergosorbent test (RAST) on worker sera using both commercial allergens prepared from grain and worksite allergens prepared from grain dust samples collected at the worksite. We found that the two types of reagents identified different populations with respect to the specificity of IgE antibodies present. The RAST assay performed using worksite allergens correlated well with skin test procedures. These results may allow us to gain better understanding of allergy associated with grain dust exposure, and document the utility of the RAST assay in assessment of occupational allergies.

Introduction

Occupational exposure to organic dusts which may contain potentially antigenic materials occurs in a wide variety of worksites and is often associated with certain occupational lung diseases (*1*). Immune responses to inhaled antigenic material have been thought to contribute directly or indirectly to the pathogenesis of numerous occupational lung diseases but the exact role of the immune system is unclear for most occupational lung diseases (*2*). Allergy, or IgE-mediated hypersensitivities, are often suspected, but clear definition of the prevalence and significance of allergic reactions to occupationally associated dusts is frequently lacking.

With respect to grain dusts, allergy has frequently been mentioned as a contributing factor, and past studies which have examined this question have reported a wide range of prevalence values up to as high as 50% of the workers studied (*3–5*). The manner in which the term "allergy" is defined, the diagnostic criteria used, the way in which the patient population was selected, and the technical methods used all greatly influence the results.

It is the technical aspects of diagnosing allergic diseases that is the subject of this report. Numerous methods exist that can be used. These range from simple questionnaires to sophisticated provocation testing using inhaled antigens (*6*). *In vivo* procedures, such as skin testing and provocation testing, are probably the most informative with respect to determining who in a particular population will react to a given dust, but it is not always possible to determine the mechanism behind that reaction. Perhaps the biggest problem with these tests is that they require skilled medical personnel to perform and interpret the tests, and in the case of provocation testing they need to be performed in a clinical setting where conditions of exposure can be carefully controlled and emergency medical facilities are available. If one defines allergy in terms of an IgE-mediated disease, then *in vitro* procedures can be used to detect the presence of IgE antibodies in the patient's serum. While such procedures allow one to detect IgE antibodies, they do not necessarily imply that the IgE antibodies are responsible for the lung disease seen, but rather that the individual has been exposed to a particular antigen and may experience an allergic reaction on re-exposure.

The *in vitro* technique most frequently utilized today is the radioallergosorbent test or RAST (*7*). We have evaluated the RAST procedure to determine if it can be applied to detect occupational allergies, and in particular we have attempted to determine the utility of using worksite dust samples in this procedure. At NIOSH we had available a panel of sera collected from grain port terminal works from the Superior–Duluth area of the United States, and grain dust samples that had been collected form the same terminals. This study population had been skin tested by the "prick" method with common aeroallergens and extracts of airborne dust (*8*). Thus we had the opportunity to compare the RAST assay using environmental samples with the skin test results and with RAST results obtained using commercially prepared allergen. Because the RAST assay

*Immunology Section, Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, Morgantown, WV 26505.

lends itself to screening large populations better than the *in vivo* methods, we hoped to determine if using the environmental samples the worksite yield similar information regarding the prevalence of allergy in this population as did the skin test procedure.

Materials and Methods

Sera Samples

Blood samples were obtained from grain terminal workers as part of a NIOSH-sponsored cross-sectional study on health effects of grain dust *(8)*. Serum from these samples were stored at –80°C until assayed.

Dust Samples

Airborne dust samples were collected during the filling of storage bins as previously described *(9)*. The dust samples were stored at 4°C until extracts were prepared. An aqueous extract of the samples was prepared by making a 10% (w/v) suspension of the dust in sterile nonpyrogenic water. The suspension was gently mixed for 1 hr at room temperature, centrifuged at 500*g* for 15 min, and the supernate recovered. The supernates were clarified by filtration through a 0.45 μm filter, and lyophilized. The resulting residue was weighed and redissolved in sterile nonpyrogenic saline at a concentration of 25 mg/mL and stored at –80°C until used. Extracts of barley, oats, rye, and spring wheat dusts were used in this study.

RAST Assay

The extracts of grain dusts were used to prepare a "solid phase allergen" for the RAST assay by reacting the extracts with cyanogen bromide-activated Sephrose-4B (CNBr Seph-4B, Pharmacia Fine Chemicals, Piscataway, NJ) according to the manufacturer's recommendations. Briefly, the extracts were adjusted to a concentration of 5 mg/mL in coupling buffer (0.1 M borate buffer, pH 8.0, containing 0.5 M NaCl). A 25-mL portion of extract was mixed with 15 mL of CNBr Seph-4B and gently mixed for 18 hr at 4°C. The beads were washed four times, the unreacted sites on the CNBr Seph-4B were blocked by reacting the beads with 1.0 M glycine as above, and following four additional washes the beads were stored in phosphate-buffered saline (0.01 M sodium phosphate, 0.1 M NaCl, pH 7.4) containing 0.2% bovine serum albumin, 0.5% Tween-20 and 0.2% sodium azide at 4°C until used. In order to estimate the amount of extract bound by the beads, the absorbance at 280 nm was recorded for each extract and for the supernates of the wash fluids. The percent of material bound by the beads was estimated by calculating the percent of material absorbing at 280 nm that was removed from the sample after reacting with beads. By this procedure we estimated that the percent of 280 nm-absorbing material bound was 74.06% of the barley extract, 32.04% of the oats extract, 72.06% of the rye extract, and 52.53% of the wheat extract.

The RAST assay itself was performed in two ways. Commercial reagents were purchased from Pharmacia Diagnostics (Piscataway, NJ), and the RAST assay using these reagents was performed according to manufacturer's recommendations. In the commercial kits, extracts of the grains are bound to cellulose discs. In addition we performed the RAST assay using the grain dust extracts bound to cellulose beads, and except for the need to pellet the beads by centrifugation during the wash steps, the assay was performed the same as the commercial assay. Patient sera (100 μL) were reacted with the beads or discs overnight with gentle agitation. The samples were washed three times; ^{125}I-labeled anti-human IgE was added to each tube and the tubes incubated overnight again. Following three additional washes the samples were counted in a gamma scintillation counter and the percent counts bound calculated. Values greater than twice the nonspecific binding control were considered positive, and for simplicity serum samples were scored as either positive or negative. Because the quantity of allergen bound to either the beads or disc was unknown and presumably different for each sample, we did not attempt to compare the assays on a more quantitative basis.

Statistical Analysis

Comparisons were made between the results obtained with commercial reagents (disc method) and those obtained using "homemade" solid-phase reagents (bead method) by both chi-square and sign test for significance.

Results

Initial experiments were conducted to determine optimal conditions for performing the RAST assay using the beads. This was accomplished by initially screening thirty randomly selected serum samples using 50μL of a 10% (v/v) suspension of beads as the immunosorbent. Based on these results we selected four serum samples, two of which were clearly positive and two which were negative. We used these sera to determine the quantity of beads required to give optimal resolution between samples containing or lacking IgE antibodies. Shown in Figure 1 is an example of the type of titration curve we obtained. From these data we concluded that 200 μL of a 10% suspension of beads is an appropriate volume to use because positive samples (sera containing IgE antibodies) could be easily identified, and negative sera gave low binding levels equal to the nonspecific binding control.

Using the two types of RAST assays, we tested 175 serum samples for IgE antibodies to either the grain or grain dust. The results are presented in Tables 1–4. The results are presented as a series of 2 × 2 tables so that the results can be directly compared. In Table 1 the results obtained with barley and barley dust are shown. Of the 174 samples assayed by both assays, 16

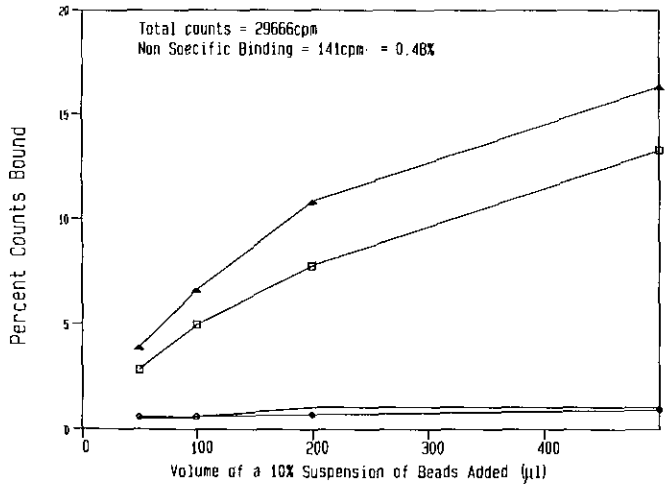

FIGURE 1. Volume of solid phase reagent (grain dust extract bound to CNBr-Seph 4B) required for the RAST assay was determined by reacting different volumes of a 10% suspension of beads with a constant volume of patient sera (100 µl). Shown is the titration of the spring wheat solid phase reagent: (▲,□) sera selected because they showed significant levels of binding in the initial screening; bottom two lines, nonreactive sera.

were found to have IgE antibodies to the grain dust, but only 6 to the grain allergen. Of the 6 that reacted with the grain, 3 reacted with the grain dust and 3 did not. When one looks at the number of discordant pairs (+/− or −/+) in Table 1, it appears that the two types of RAST assays were measuring two different IgE specificities. Statistical analysis by the sign test showed that

Table 1. Comparison of RAST results obtained using commercial reagents (disc) with environmental reagents (beads) on extracts prepared from barley dust.

	Beads		
Disc	Positive	Negative	Total
Positive	3	3	6
Negative	13	155	168
Total	16	158	174

Table 2. Comparison of RAST results obtained using commercial reagents (disc) with environmental reagents (beads) on extracts prepared from oats dust.

	Beads		
Disc	Positive	Negative	Total
Positive	3	4	7
Negative	16	151	167
Total	19	155	174

Table 3. Comparison of RAST results obtained using commercial reagents (disc) with environmental reagents (beads) on extracts prepared from rye dust.

	Beads		
Disc	Positive	Negative	Tatal
Positive	6	6	12
Negative	5	148	153
Total	11	154	165

Table 4. Comparison of RAST results obtained using commercial reagents (disc) with environmental reagents (beads) on extracts prepared from wheat dust.

	Beads		
Disc	Positive	Negative	Total
Positive	4	5	9
Negative	19	147	166
Total	23	152	175

there was a significant level of discordance between the two types of assays ($p<0.05$). If one examines the other three tables in the same manner, there is significant discordance between the results of the two assays for each of the grain or grain dust pairs.

We compared the results obtained by the RAST procedures with those obtained by skin testing of the same population of grain workers. The comparisons are shown in Table 5. The RAST assay using extracts of environmental samples coupled to cellulose beads revealed a higher prevalence of positive reactors than did the commercial disc methods for three of the four grains. If one compares these results with the skin test data the "bead" method compared more favorably than the disc method. For example, by both the bead RAST and skin testing, wheat had the highest prevalence and rye the lowest prevalence of positive reactors. In addition, the total prevalence of reactors (i.e., those individuals reacting with one or more of the samples) was not statistically different between the "bead" RAST and skin testing but the "disc" RAST and skin testing were significantly different ($p<0.025$). We had only summary data available from the earlier study so we could not make a direct comparison on a subject by subject basis between the "bead" RAST and skin test results.

Discussion

The purpose of the present study was twofold. First, we wanted to determine if extracts of environmental samples could be used to prepare RAST reagents that could be used to define the prevalence of IgE antibodies to environmental allergens in particular worker populations. In addition, we hoped to determine if the RAST assay performed using the environmental reagents offered any advantage over RAST assays using commercially available reagents. The results obtained indicated that the RAST assay can be easily modified to use en-

Table 5. Comparison of the prevalence of allergy in grain handlers as determined by RAST or skin test assays.

Dust sample	Disc[a]	Beads[a]	Skin testing[a,b]
Barley	3.45	9.20	8.9
Oats	4.02	10.92	5.9
Rye	7.27	6.67	5.6
Wheat	5.14	13.14	9.5
Rx with one or more	9.71	19.43	26.6

[a] For RAST, $n = 175$; for skin testing, $n = 305$.
[b] Data from Rankin (*8*).

vironmental samples as the source allergen. The finding that the modified RAST detected IgE antibodies in a greater percentage of the exposed worker, and that these results correlated with the skin test results better than the commercial kit is not surprising. The environmental samples would be expected to have more of the relevant allergens. However, when attempting to use environmental samples, several points need to be considered before one can conclude that an assay like the RAST is acceptable. Because the chemical nature of the suspect allergen is unknown, it is necessary to determine if the allergen can be bound to the solid phase particles (cellulose beads). In the present study we knew from the earlier work that the allergens were contained in the aqueous extracts of the dusts. We did not attempt to chemically characterize the allergens present, but assumed that since they were derived from organic material they would contain reactive groups that would allow them to be bound by the CNBr-Seph4B. That material absorbing light at 280 nm was adsorbed by the beads supports this assumption. To prove that the relevant allergens were bound to the beads would require testing extracts' supernates after reacting with the CNBr-Seph4B in a skin test assay to insure that all of the allergens were removed. We did not have the opportunity to do this. However, because we were able to identify a significant number of positive reactors at least some if not all of the allergens were bound to the cellulose beads. Another important consideration in performing the RAST assay using the beads is to determine an optimal volume of beads to use. If the volume of beads is too low, then IgG antibodies which may be present in the sera can compete with the IgE antibodies and thus reduce the apparent amount of IgE bound by the beads (*10*). Conversely, as the volume of beads is increased, then the level of nonspecific binding also increases and this can reduce the sensitivity of the assay. Therefore, it is necessary to carry out preliminary experiments to insure that the relative concentrations of the reactants is appropriated so that meaningful results will be obtained.

Perhaps the most difficult aspect of developing a RAST assay for environmental and occupational allergens is the lack of standardized positive controls to insure that the assay works. In the present study we simply screened a number of the serum samples until we identified two which gave strong positive reactions, and used those sera to then standardize the assay. However, because we had no reference sera, we did not attempt to rank the positive reactors on a 1+ to 4+ scale as is done in the commercial RAST assay. Rather, we chose simply to identify the sera as positive or negative, using a value of twice the nonspecific binding control as the cutoff point between positive and negative. Other investigators have used this as the criterion to identify positive reactors in the RAST assay (*11*). In addition, we examined the frequency distribution of the data and found that the percent counts bound values for the sera we identified as negative clustered and were normally distributed around the nonspecific binding values. The twice negative control value was greater than two standard deviations away from the mean of the negative values, indicating that our positive reactors were a distinct subpopulation. Thus we feel confident that the RAST as we performed it was identifying those sera which contained significant levels of IgE antibodies to the grain dust allergens. Because of all the constraints mentioned above, we would be most likely to be underestimating the actual prevalence of these antibodies.

The value of doing the RAST assay with extracts of dust samples obtained from the local environment is demonstrated in Table 5. Although there was not complete agreement between the results obtained with the bead RAST and the skin test procedure, these assays agreed better than the commercial RAST (disc) did with either method. The skin testing was done with extracts prepared from dust samples collected at the work site, but these were not the same samples of dust or extracts we used in the RAST assay. One would expect that there would be a greater probability of the same allergens occurring in the extracts used for skin testing and the ones we prepared than in the commercial preparations. Presumably the commercial preparations were made from the whole grain and not just the dust fraction of the grain. This study demonstrates that using environmental samples as a source of allergen for the RAST assay allows one to obtain prevalence data comparable to what one would obtain by skin testing. When possible, skin testing is still the preferable method for obtaining information about the prevalence of allergy in a defined population (*12*). However, skin testing does require trained medical personnel to prepare extracts, administer the test, and interpret the results, and there is some risk, albeit small, that adverse reactions may occur that require immediate medical attention. Particularly in the work site environment, facilities may not be available for skin testing. If blood samples can be obtained then the RAST assay can be used as an alternative to skin testing. Although not part of the present study, the major advantage of the RAST assay may lie in the use of the assay to monitor the isolation and identification of allergen in defined environments (*13,14*).

In conclusion, the results of this study demonstrate that aqueous extracts of grain dusts can be used as a source material for the RAST assay. Using this assay we found that the prevalence of IgE antibodies to grain dust antigens was significantly higher than the prevalence of IgE antibodies to grain antigens, and that the prevalence of IgE antibodies to grain dust antigens correlated well with skin test results. The presence of IgE antibodies to occupationally associated antigens suggests that these workers may be at a greater risk of developing respiratory problems, but it is unclear if the presence of such antibodies contribute to the pulmonary diseases associated with grain dust.

The authors thank Mrs. Beverly Carter for excellent secretarial assistance.

Mention of company names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

REFERENCES

1. Parks, W. R. Disorders caused by organic agents. In: Occupational Lung Disorders. Butterworths, London, 1982, pp. 359–414.
2. Burrell, R. Immunology of occupational lung diseases. In: Occupational Lung Diseases, 2nd Ed. (W. K. C. Morgan and A. Seaton, Eds.), W.B. Saunders, Philadelphia, 1984, pp. 196–211.
3. doPico, G.A., Reddan, W., Flaherty, D., Tsiatis, A. Peters, M. E., Rao, P., and Rankin, J. Respiratory abnormalities among grain handlers. Am. Rev. Resp. Dis. 115: 915–927 (1977).
4. Warren, P., Cherniack, R. M., and Tse, K. S. Hypersensitivity reactions to grain dust. J. Allergy Clin. Immunol. 53: 139–149 (1974).
5. Skoulas, A., Williams, N., and Merriman, J.E. Exposure to grain dust. II. A clinical study of the effect. J. Occup. Med. 6: 359–372 (1964).
6. Johansson, S.G.O. *In vitro* diagnosis of reaginmediated allergic diseases. Allergy 33: 292–298 (1978).
7. Wide, L., Bennich, H., and Johansson, S. G. O. Diagnosis of allergy by an *in vitro* test for allergen antibodies. Lancet ii: 1105–1107 (1967).
8. Rankin, J. R. National Institute of Occupational Safety and Health, Contract Report 210-76-0175 Morgantown, WV (N.D.)
9. Peach, M. J., Olenchock, S. A., Sorenson, W. A., and Major, P. C. Relevance of grain dust collection techniques to respiratory disease studies. In: Occupational Pulmonary Disease. Focus on Grain Dust and Health (J.A. Dosman and D.J. Cotton, Eds.), Academic Press, New York, 1980, pp. 507–512.
10. Zimmermann, E. M., Yunginger, J. W., and Gleich, G. J. Interference in ragweed pollen and honeybee venom radioallergosorbent tests. J. Allergy Clin. Immunol. 66: 386–393 (1980).
11. Gleich, G. J., Aldolphson, C. R., And Yunginger, J. W. The mini-RAST: comparison with other varieties of the radioallergosorbent test for the measurement of immunoglobulin E antibodies. J. Allergy Clin. Immunol. 65: 20–28 (1980).
12. Norman, P. S. Skin testing. In: Manual of Clinical Immunology, 2nd Ed. (N. R. Rose and H. Friedman, Eds.), American Society for Microbiology, Washington, DC, 1980, pp. 789–793.
13. Agarwal, M. K., Yunginger, J. W., Swanson, M.C., and Reed, C. E. An immunochemical method to measure atmospheric allergens. J. Allergy Clin. Immunol. 68: 194–200 (1981).
14. Twiggs, J. T., Agarwal, M. K., Dahlberg, M. J. E., and Yunginger, J. W. Immunochemical measurement of airborne mouse allergens in a laboratory facility. J. Allergy Clin. Immunol. 69: 522–526 (1982).